



Phylogenetic analysis of ornamental Pomacentridae from the southern coast of West Java

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Abstract. Pomacentridae has a wide distribution in Indonesia waters. Research on species richness of Pomacentridae has been carried out in some areas in Indonesia. However, there are no data about the relationships of Pomacentridae on the southern coast of West Java. This research aims to know the evolutionary kinship of Pomacentridae ornamental fish in Ujung Genteng and Taman Manalusu Beach, West Java. This research used a survey method with a judgment sampling technique. The parameters measured were the consistency index (CI) and character evolution of the resulting phylogenetic tree. Cladogram reconstructed based on maximum parsimony algorithm with 1000 bootstrap replicates in PAUP software. The result showed that the tree had a CI value of 0.6000 which indicated low homoplasy. Pomacentridae formed a monophyletic group when they compared with Chaetodon collare as outgroup. The Pomacentridae clade divided into two subclades. The first subclade consisted of Abudedefduf indicus as a basal species, then Neoglyphidodon bonang and Plectroglyphidodon lacrymatus have a closed kinship and diverged after Dascyllus trimaculatus, and then Abudedefduf notatus and Abudedefduf sexfasciatus have a close kinship and diverged after Abudedefduf vaigiensis. The second subclade referred to as a basal group, and within this subclade, Chrysiptera brownriggii has a closely related to Chrysiptera unimaculata and diverged after Chromis opercularis.

Keywords: Cladistics, damselfish, meristic, morphometric, Pomacentridae

Introduction

Ujung Genteng in Sukabumi Regency and Taman Manalusu in Garut Regency are among the southern coastline in West Java Province. Both coastal regions hosted great natural resources, such as reef areas, seagrass beds, mangrove forests, and fisheries resources, including marine ornamental fish (BPLHD, 2008).

Marine ornamental fish have a small and colorful body that attracts high interest from hobbyists for aquarium attraction. The phenomena lead to the improvement of market demand on that commodity (Umar and Ahmad, 2008). As a consequence, natural populations of marine ornamental fish are continuously exploited since the aquarium trade of this commodity mostly supported by nature fishing (Hoeksema, 2007). The phenomena are widespread across Indonesia (Reksodihardjo-Lilley and Lilley, 2007; Nijman, 2010) including the south coast of West Java.

Pomacentridae is among marine ornamental fish attain high interest from the hobbyist. This fish is well-known with the common name of *damselfish* (Lee, 2017). Fish species from family widely distributed across tropical marine ecosystems. Some species found inhabit in the water ecosystem with medium climate and brackish water, but most of them live in shallow water at coral reef ecosystems (Cooper *et al.*, 2009).

Pomacentridae is an abundant fish family within Perciformes with total species of approximately 340 (Jang-Liaw *et al.*, 2002). Among 340 species, a total of 165 species



described from Indonesia (Froese and Pauly, 2019). High species diversity within Pomacentridae has led to vast morphological variation. Morphological variations among species within Pomacentridae are interesting to be studied, especially on their phylogeny. Morphological observation in meristics and morphometric measurement are a primary method for species identification and phylogenetic analysis (Aguilar-Medrano *et al.*, 2011; Liu *et al.*, 2013).

Phylogeny is a prediction about evolutionary relationships among organisms, either fossil or living organisms. According to (Michu, 2007), the importance of phylogeny information among organisms is providing a comprehensive understanding of the origin and evolutionary history of certain species. Also, (Dharmayanti, 2011) noted that phylogenetic data provide an evolutionary history of species, character changes, and can be used to estimate the difference of one generation to other generations.

Relationships among organisms can be estimated from phenetic and phylogenetic approaches (Rokas *et al.*, 2003; Posada and Buckley, 2004;). The logic approach to relationships analysis of living things based on their evolutionary history is phylogenetic or cladistics analysis (Dharmayanti, 2011). During phylogenetic analysis, the evolutionary history of organisms identified from their character changes. Similar characters are the basis for relationships among species. The most common statistical tool used in phylogenetic analysis is the maximum parsimony algorithm. The maximum parsimony algorithm results in the minimum number of character change on the phylogenetic tree and leads to low homoplasy (Yang and Rannala, 2012).

Previous study showed the phylogenetic relationships among species under Pomacentridae either based on morphology characters (Aguilar-Medrano *et al.*, 2011; Aguilar-Medrano, 2013; Frédérick *et al.*, 2013; Liu *et al.*, 2013) or molecular characters (McCafferty *et al.*, 2002; Quenouille *et al.*, 2004; Timm *et al.*, 2008; Drew and Barber, 2009; Sorenson *et al.*, 2014;). However, no scientific publication was available for the phylogenetic relationship among pomacentrid species from the southern coast of Ujung Genteng and Taman Manalusu, West Java. Phylogenetic relationships data is among vital information for further study in Pomacentridae and management of sustainability of Pomacentridae on the south coast of West Java with special emphasis to Ujung Genteng Sukabumi Regency and Taman Manulusu Garut Regency.

This study aimed to obtain data on the evolutionary relationships among species of Pomacentridae from the southern coast of Ujung Genteng Sukabumi and Taman Manalusu Garut, West Java with special reference to ornamental species.

Materials and Methods

Research location and time

We focused on ornamental pomacentrids that traded in Ujung Genteng Sukabumi and Taman Manalusu Garut. According to the research focus, fish samples collected from the first middleman both in Ujung Genteng Sukabumi and Taman Manalusu Garut (Figure 1). The samples collected in February and March 2018.

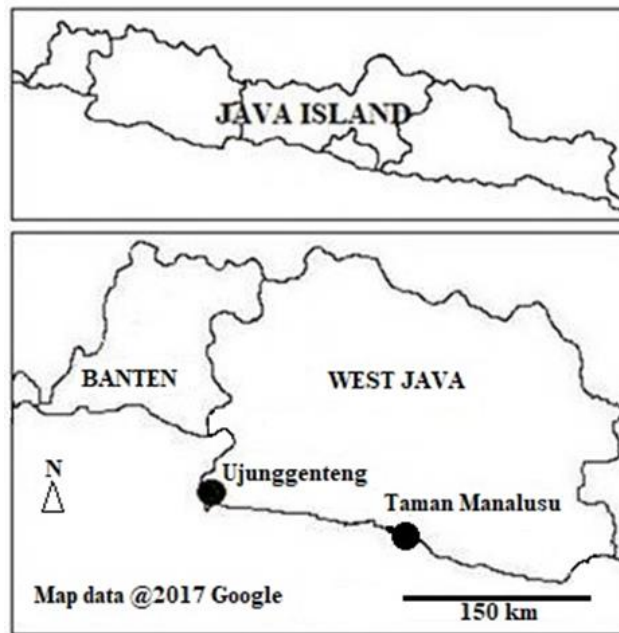


Figure 1. Schematic map of West Java with Ujung Genteng and Taman Manalusu as sampling sites

Fish handling

Fresh individuals of each morphotype photographed using 8 megapixels automatic Camera. This step is carried out to obtain an original picture of the color and color pattern of each morphotype since both characters are among key characters for species determination. All samples were preserved in 70% ethanol. Upon arrival in the laboratory, the old ethanol was replaced by the fresh one for permanent preservation. Meristic characters, such as fin rays and spines, were counted manually with the help of a magnification lens. Morphometrics characters, like standard and head length, were measured using caliper with an accuracy of 0.001 cm.

Data analysis

The taxonomic status of each morphotype was obtained from identification and determination by referring to Allen and Erdmann (2012). The validity of obtained scientific names was referred to as the database available in FishBase (Froese and Pauly, 2019). Phylogenetic analysis was performed through phylogenetic tree or cladogram reconstruction. The cladogram was reconstructed based on 24 morphological characters which divided into three groups of characters; the ratio between two morphometrics measure, meristic, and general morphology performance. Morphometric characters refer to Ducic (2005). Detail characters used for phylogenetic analysis summarized in Table 1.

All used morphology characters were transformed into multistate quantitative characters. Three different character states were gained from the mathematical calculation in excel software. Each character's state was symbolized by different notations; e.g. 0, 1, and 2, respectively. The symbol 0 represents primitive characters in outgroup species. The symbols 1 and 2 represent derived characters of fish samples, detail meaning of the symbols presented in Table 2.

Table 2. The meaning of transformation symbols

Symbol	Meaning
0	Small or less or absent
1	Medium or 2 lines or short or black spots



The matrix of character symbols saved in three different nexus files; namely taxa block, characters block, and PAUP block. The cladogram was reconstructed using the maximum parsimony algorithm in PAUP 4.0 software (Swofford, 2002) with 1000 bootstrap replications. The maximum parsimony algorithm is the most reliable algorithm for evolutionary relationship analysis compared to other algorithm since it considers evolutionary processes and the evolution probability of each character. Tree branching polarity inferred from outgroups comparison. *Chaetodon collare* used as outgroup species because it shared more primitive (plesiomorphic) characters with Pomacentridae since *C. collare* and Pomacentridae belong to a single order Perciformes.

The variable was evolutionary relationships among ornamental pomacentrids fish. The parameters consisted of a consistency index (CI), monophyly, and character alteration among species. The consistency index was automatically calculated based on a mathematic calculation by the PAUP 4.0 software (Swofford, 2002). Monophyly of pomacentrid clade and character mutation among species were analyzed descriptively based on branching pattern and character alteration shown in the cladogram.

Table 1. Morphological characters used in the phylogenetic analysis of pomacentrid fish

No	Character	Annotation
1	Lc:Ls	Ratio head length to standard length
2	Ld:Ls	Ratio dorsal fin length to standard length
3	La:Ls	anal fin length to standard length
4	Lp:Ls	pectoral fin length to standard length
5	Lv:Ls	ventral fin length to standard length
6	Hmin:Ls	minimum body height to standard length
7	Hmax:Lc	maximum body height to head length
8	Hmax:Ld	maximum body height to dorsal fin length
9	Hmax:La	maximum body height to anal fin length
10	Hmax:Lp	maximum body height to pectoral fin
11	Hmax:Lv	maximum body height to ventral fin length
12	Hmax:Hmin	maximum body height to minimum body height
13	O:Lc	eye diameter to head length
14	Lpo:Lc	post ocular length to head length
15	Lio:Lc	interocular length to head length
16	PO:Lc	pre ocular length to head length
17	Sdf	Soft dorsal fin rays
18	Sdc	Soft caudal fin rays
19	Sda	Soft anal fin rays
20	Sdp	Soft pectoral fin rays
21	Lls	Linea lateral scales
22	VI	Vertical line
23	HI	Horizontal line
24	S	spot

Results

During the study, a total of ten species of Pomacentridae was traded on the southern coast of Ujung Genteng Sukabumi and Taman Manalusu West Java, Indonesia. The evolutionary relationships among those ten species illustrated in a cladogram as presented in Figure 2. The cladogram has 75 steps length with the consistency index was 0.600.

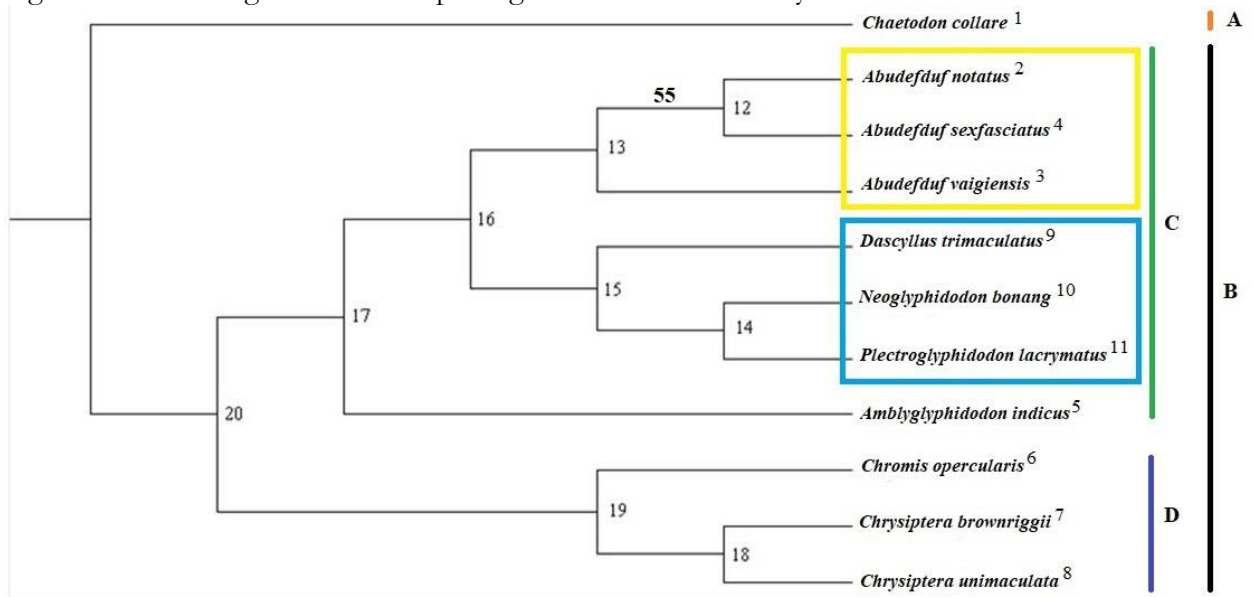
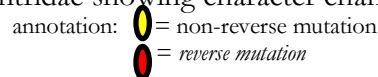


Figure 2. Cladogram of the evolutionary relationship among ten Pomacentridae species from Ujung Genteng and Taman Manulusu. Remarks: 1-20 = nodus number

All species within Family Pomacentridae from both Ujung Genteng and Taman Manalusu formed a monophyletic group or clade (B) compared to *Chaetodon collare* as the outgroup species. Pomacentrid clade further divided into two sub-clades C and D. The subclade C formed by seven pomacentrid species. The subclade D consisted of three species, namely *Chromis opercularis*, *Chrysiptera brownriggii*, and *Chrysiptera unimaculata*. Subclade C further divided into basal species and two sub-subclades. Basal species occupied by *Amblyglyphidodon indicus*. The first sub-subclade (yellow squares) formed by *Abudefduf notatus*, *Abudefduf sexfasciatus*, and *Abudefduf vaigiensis*. The second sub-sub clade (light blue squares) consisted of *Dascyllus trimaculatus*, *Neoglyphidodon bonang*, and *Plectroglyphidodon lacrymatus*.

It also observed in Figure 2 that *A. notatus* and *A. sexfasciatus* are evolutionary close related and diverged after *A. vaigiensis*. In the second sub-subclade, *N. bonang* is evolutionary close related to *P. lacrymatus* and evolved after *D. trimaculatus*. The second subclade (C) referred to as a basal group where *C. brownrigii* phylogenetically close related to *C. unimaculata* and diverged after *C. opercularis*.

The cladogram in Figure 3 illustrated the character mutations that occurred during pomacentrid species formation in evolutionary times. It was observed that there are two types of character changes during pomacentrid evolution, namely, reverse (red ovoid) and no reverse (yellow ovoid). Character mutation that separate or unite of closely related species or group of species illustrated in Figure 3.



Similar results of a low level of homoplasy on phylogenetic analysis using morphometric characters also observed by Nabila *et al.* (2019) who also reported low homoplasy on the morphological character of Acanthuridae. A low homoplasy level was also



found in Labridae (Pambudi *et al.* 2019). Furthermore, several studies also found a low level of homoplasy in morphological characters of terrestrial organisms. For instance, Klingenberg and Gidaszewski (2010) observed low morphological homoplasy on *Drosophila melanogaster*, while similar low-level of homoplasy were also reported by Klußmann-Fricke *et al.* (2012) in Scorpion. The comparison to other studies showed that the result of our study had a different level of homoplasy from a study of Mueller *et al.* (2004) who reported high-level homoplasy in morphological characters of plethodontid Salamander. The difference could be due to that both studies used different animal group although used similar statistical tools. Within this report, we used fish samples, whereas (Mueller *et al.*, 2004) used salamander.

The low homoplasy also indicates that the similarity observed in all ten species of pomacentrid referred to as character homology among species from the same ancestor. This similarity referred to as shared primitive characters of descendants under a particular ancestor or synapomorphy (Szucsich and Pass, 2008).

Homoplasy indicates convergent evolution between two or more lineages. In this situation, two or more different species are highly similar in a particular character. According to (Klingenberg and Gidaszewski, 2010), homoplasy results in analog characters among lineages. It had further explained that homoplasy is the appearance of a particular character or identical character within two or more lineages that occurred independently.

Our study also proved that the used morphological characters showed low homoplasy. It was due to the characters were selected carefully. Low homoplasy was also due to the characters was weighted and the maximum parsimony algorithm was used. According to Barbancon *et al.* (2013), morphology character naturally showed high homoplasy. However, screening of morphological character for phylogenetic analysis results in low homoplasy. Weighted character led to low homoplasy than unweighted ones. Moreover, the maximum parsimony algorithm might decrease the level of homoplasy compared to the unweighted pair group method with arithmetic mean (UPGMA).

The monophyly of pomacentrid samples indicates that phylogenetic classification supports phenetic classification. It was noted by Littlewood *et al.* (2004) that classification based on evolutionary relationships might support traditional classification based on the total number of similarities in morphological characters. The monophyly of closed related species in Pomacentrid also reported in *Chromis* and *Azurina* (Aguilar-Medrano *et al.*, 2011; Aguilar-Medrano, 2013; Liu *et al.*, 2013).

The monophyly of species under study as reported in this study and the studies from Aguilar-Medrano *et al.* (2011), Frédérich *et al.* (2013), and from Aguilar-Medrano *et al.* (2011) provide strong evidence the reliability of morphological characters in phylogenetic studies as long as selective characters and outgroups species used. It was reported that the selection of closed related taxa in the phylogenetic study might improve the polarity of branching topology of a cladogram (Rohland *et al.*, 2007).

If we come into detail in Figure 3, the separation of *C. collare* from the Pomacentridae group proved that *C. collare* was the primitive taxon. *C. collare* separated from Pomacentridae by four plesiomorphic characters. Those characters were character number 5 (ratio between anal fin length and standard length), 11 (ratio between maximum body height and ventral fin length), 18 (soft caudal-fin rays), and character number 20 (soft pectoral fin rays).

Node number 20 was a synapomorphic with node number 17 based on character number 3 (comparison between standard length and anal fin length), 7 (comparison between body height and head length), and character number 10 (ratio between body height and pectoral fin length). Node 17 is a synapomorphic with node 16 based on character number 5 (comparison between standard length and ventral fin length), and character number 12 (comparison between height and tail stem height).



A. notatus, *A. sexfasciatus*, and *A. vaigiensis* were a monophyletic group. They combined by synapomorphic character number 9 (comparison between height and anal fin length), character number 13 (comparison between head length and eye diameter), character number 21 (number of scales in lateral line), and character number 22 (body pattern in the form of vertical lines on the body).

A. notatus was a sister species *A. sexfasciatus* based on shared characters (synapomorphic) on character number 3 (comparison between standard length and anal fin length), character number 4 (comparison between standard length and pectoral fin length), character number 10 (comparison between height and pectoral fin length) and character number 15 (comparison between head length and intra-ocular length).

A. notatus has apomorphic characters based on character number 5 (comparison between standard length and ventral fin length), character number 7 (ratio between height to head length), character number 8 (comparison between height and dorsal fin length), character number 11 (comparison between height and ventral fin length), and character number 19 (number of soft anal fin fingers).

Apomorphic characters owned by *A. notatus* were character number 3 (comparison between standard length and anal fin length), character number 6 (comparison between standard length and tail stem height), and character number 9 (comparison between body height and anal fin length). Other apomorphic characters of *A. notatus* were character number 12 (ratio between height and tail stem height), character number 14 (comparison between head length and postocular length), and character number 16 (comparison between head length and pre-ocular length).

D. trimaculatus, *N. bonang*, and *P. lacrimatus* are monophyletic groups based on shared characters (synapomorphy) on character number 7 (ratio of height to head length), character number 17 (number of soft dorsal fin radii), character number 19 (number of anal fin soft fingers), character number 20 (number of pectoral fin soft fingers), and character number 24 (body pattern in the form of spots).

D. trimaculatus had apomorphic characters on character number 3 (comparison between standard length and anal fin length), character number 7 (ratio between height and head length), character number 11 (ratio between height and ventral fin length), and character number 12 (comparison between height and tail stem height). *D. trimaculatus* followed by the development of *N. bonang* and *P. lacrimatus* who are strictly related and synapomorphic based on character number 15 (comparison between head length and intra-ocular length).

N. bonang was apomorphic based on character number 4 (comparison between standard length and pectoral fin length), character number 9 (comparison between height and anal fin length), character number 12 (ratio between height and tail stem height), and character number 16 (comparison between head length and pre-ocular length). Apomorphic characters of *N. bonang* were character number 21 (number of scales in lateral line), character number 23 (body pattern in the form of horizontal lines), and character number 24 (body pattern in the form of spots). *P. lacrimatus* was apomorphic based on character number 6 (comparison between standard length and tail stem height), character number 10 (comparison between height and pectoral fin length), character number 13 (comparison between head length and eye diameter), and character number 17 (number of dorsal fin soft radii).

A. indicus is apomorphic based on character number 1 (comparison between standard length and head length), character number 4 (comparison between standard length and pectoral fin length), character number 6 (comparison between standard length and tail stem height), character number 13 (comparison between head length and eye diameter), and character number 14 (comparison between head length and postocular length).



C. opercularis, *C. brownriggii*, and *C. unimaculata* form a monophyletic group based on common ancestor characters (synapomorphies) on character number 15 (comparison between head length with intra-ocular length), character number 19 (number of soft fin radius of anal), and character number 24 (spot body pattern). *C. opercularis* was apomorphy based on character number 6 (comparison between standard length and tail stem height), character number 11 (comparison between height and ventral fin length), and character number 12 (comparison between height and tail stem height). The formation of sister species *C. brownriggii*, and *C. unimaculata* occurred by the presence of synapomorphic characters in character number 1 (comparison between standard length and head length), character number 4 (comparison between standard length with pectoral fin length), and character number 13 (comparison between head length and eye diameter).

C. brownriggii was apomorphic based on character number 2 (comparison between standard length and dorsal fin length), character number 3 (comparison between standard length and anal fin length), character number 9 (comparison between body height and anal fin length), and character number 14 (comparison between head length and postocular length). *C. brownriggii* has apomorphic characters on character number 18 (number of soft caudal fin radii), character number 20 (number of pectoral fin soft radii), and character number 22 (body pattern in the form of vertical lines on the body).

C. unimaculata apomorphic based on character number 4 (comparison between standard length and pectoral fin length), character number 6 (comparison between standard length and tail stem height), character number 15 (comparison between head length and intra-ocular length), character number 19 (number of anal fin soft fingers), and character number 23 (body pattern in the form of horizontal lines).

Species under Pomacentridae formed a monophyletic clade because they have derived characters that mutated from primitive characters in most common ancestor which closely related to *C. collare*. A similar situation also observed in all closely related groups and sister taxa. Each species separated one to the others by having specific characters that were distinct from other species. For instance, *D. trimaculatus* has characters number 7, while *N. boning* does not have that character and vice versa (character number 24 was present in *N. bonang* but absent in *D. trimaculatus*, and so forth). It has noted previously by Szucsich and Pass (2008) that a monophyletic group created by taxa that have new characters. Moreover, Mueller *et al.* (2004) explained that a clade formed if more than one taxon shared derived characters which altered from primitive characters that present in common ancestor.

Conclusion

The obtained cladogram was the most parsimonious tree with short steps, high consistency index and a high number of non-reverse mutation. Species under Pomacentridae formed a monophyletic clade when compared to *C. collare* as *outgroup*. Pomacentridae clade divided into two subclades. The first subclade formed by *A. indicus* as basal species, *P. lacrymatus*, *N. bonang*, *D. trimaculatus*, *A. notatus*, *A. sexfaciatus*, and *A. vaigiensis*. The second subclade referred to as a basal group and consisted of *C. brownriggii*, *C. unimaculata*, and *C. opercularis*.

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